Opponents review of the PhD Thesis Mgr. Marcel Štofik

**Biosensors for Environmental Monitoring and Biomedical Applications**

Thesis deals apparently with the long term tracted problem of biosensors. The potential advantage of biosensors is obvious. Any chemical analysis is targeted, it can analyse only a certain class of chemical compounds which may be detected by a particular assay. Biosensors have the potential advantage to target a whole class of compounds, i.e. all inhibitors of a particular enzyme. The lack of knowledge of chemical identity of the detected compound is compensated by detection of biological – or more often biochemical – activity.

Work on biosensors is quite extensive over many decades. Most of the hurdles on the way towards its practical use are technical. There are problems with isolation of enzymes and enzyme complexes, efficient fixation of enzymes at surfaces of detecting systems and efficient and controlled delivery of analysed material. All these problems are discussed by the candidate in the introduction to the thesis.

The problem on which the candidate was working is rather long-term, first results have been obtained in 1996, first published paper dates back to 1998. The idea of preparation of photosystem II biosensor is rather obvious. Upon illumination photosystem II transports electrons and this process thus may be electrochemically detected. The success of biochemistry in 1980´s and 1990´ lead to assumption that enzymes may be isolated, stabilised on a suitable surface and otherwise treated as surface-bound catalysts. In 1998 the photosystem II – based biosensor was published and the avenue towards its practical use seemed to be straightforward. Now, in 2012, there still does not seem to be any product near the market use. Quite the opposite, the presented thesis lists a number of methods of alternative stabilisation of the enzyme at the electrode surface which implicitely indicates that the previous method is insufficient.

Some of the results regarding the photosystem II based sensors are presented in the thesis in yet unpublished paper. It is the Paper one, where candidate is the first author and it is listed in the thesis as Stofik et al., 2008. In the list of publication this paper is mentioned as the one paper to which the candidate contributed mostly. I have to admit that listing the key unpublished paper as the unpublished for four years is at minimum not very tactical. The results given in the article show high variability and, perhaps, even signs of qualitative difference even in the case of best chosen preparation as at figure 5 at the page 66. Furthermore, authors themselves report anomalous behaviour. This indicates that that any simplification by using statistics based on assumption of normal distribution as at the figure 2 is not fully justified. More information and samples of primary data will be needed to evaluate this problem. Since in this case I also can not refer to the wisdom of the anonymous reviewer, I take the liberty to refrain from consideration of this publication in results of this thesis.

Fortunately the candidate has participated in other extensive research efforts. He is the first author of the paper II, where he contributed significantly to characterisation of dendrimer-enscapsulated silver nanoparticles. In this way is satisfied the first requirement for defence of the thesis. In this paper there is a combination of structural, electrochemical and spectroscopic methods which proves candidates profesionality in biophysical methodology.

The candidate has also participated in other technical developments, namely in development of the biotinylated silver dendrimer nanocomposites (Paper III). He is also first author of the utility model for the holder of the microfludic chip (Paper IV and V).

Finally. I particularly recognise the Paper VI. This includes theoretical design of the magnetic microbead separator, design of the lithographic mask according to which it may be constructed as well as some early experiment testing it. The later article is readable and comprehensible, it includes sufficient details to understand the content and adequately represents experiments.

Papers relevant for the thesis, actually, do not deal with the subject of biosensors in particular. They cover a range of technical development of which some may be used in biosensor development but have much broader significance. This makes the theses rather interesting for general public, in fact quite unique and very valuable. Also, as judged from contents of published paper, the candidate acquired quite deep insight into the problem.

In conclusion, I admit the progress which the candidate has made during the years of work at his PhD theses. The progress from a student who refused to take the examination in physical biology for not being sufficiently prepared to the person which is able to collaborate on design microfluidic devices and magnetic separators is admirable. The thesis at the end is very technical, it is based mainly on technical developments. I admit this personally since I have also gone through the personal development from the scientist by heart to development of techniques over the last few years. It is clear that experimenters less and less understand their techniques and trust to the producers. It is rather important to go at certain stage of the professional development through design of all parts of your experiment, the engineering one as well as the manufacturing one. Marcel Štofík is well prepared for scientific career in the future.

At this place, I recommend awarding of the PhD title to the candidate.

To the content of the thesis I have following questions to the candidate:

1) In the paper II at Figure 3, you show electron micrographs of nanoparticles. Did you try to resolve shapes of the particles? What were the shapes? Which influence would the diversity of objects have on spectroscopic and electrochemical properties of the material?

2) In the paper III at figure 5 and in part 3.4 you show the surface plasmon resonance signal of the binding of biotinylated silver-dendrimer nanocomposites to avidin. Biotin-avidin complex is know to be one of the strongest complexes in biological systems. It is used as standard for testing the binding in biological experiments. Did you make comparison of binding of your conjugate and of pure biotin? Was the binding in any way affected by the particle size heterogeneity which you report in this article? If not, how do you explain it?

3) In the paper VI you report the trapping of magnetic beads by your microcoil system. It may be also understood as visualisation of your theoretical prediction of the distribution magnetic power, the more beads bound, the more

beads trapped. Is this a correct assumption or not? If not, why, what is the difference? If yes, does the experiment confirm the prediction? Does any other visualisation method of magnetic force exist to confirm your theoretical predictions?

V Sundalsøra 14. 8. 2012

doc. RNDr. Dalibor Štys CSc.

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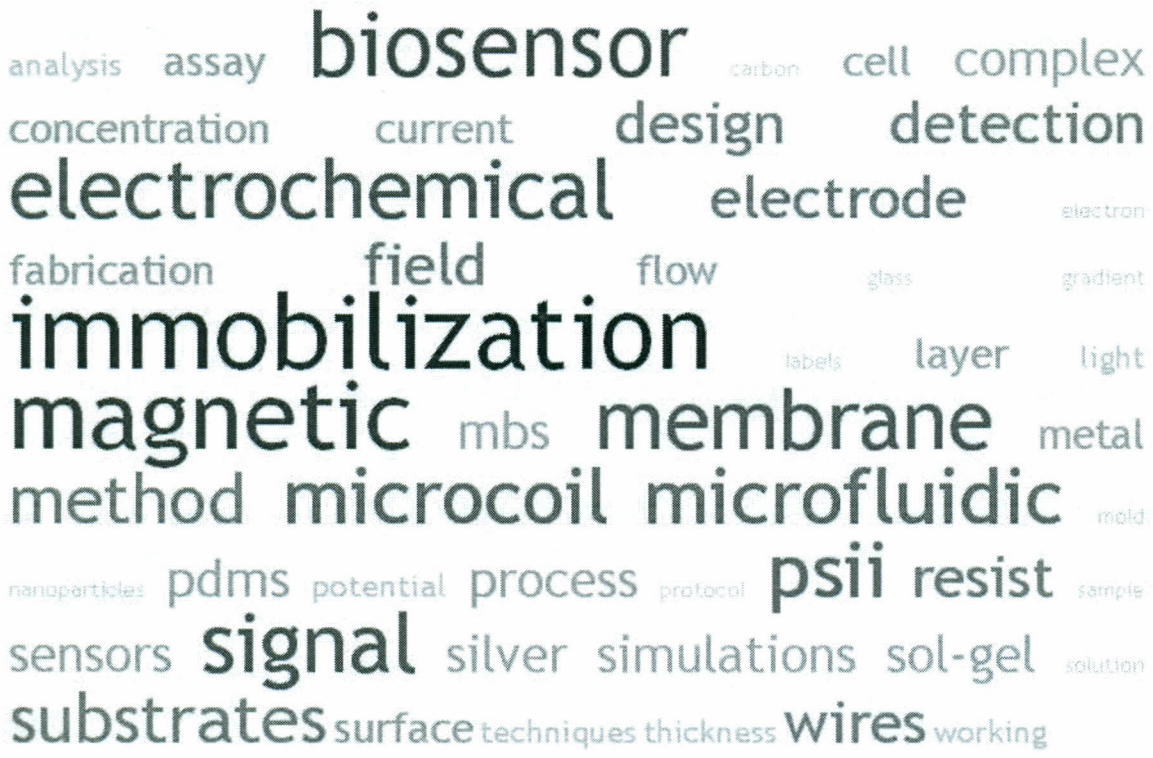
**Referee's report** of the PhD thesis by Mr. **Marcel Štofik** and entitled

**"Biosensors for Environmental Monitoringand Biomedical Applications"**

Opponent Review by Roberto Pilloton

The thesis deals very interesting points about the preparation of biosensors for environmental monitoring and biomedical applications as well as immobilization and stabilization of biological molecules,separation of immobilized molecule by meand of magnetic field, synthesis and fabrication of sensitive electrochemical labels based on silver nanoparticles, electrochemical detection and microfluidics. Here is reported a *"world cloud"* of the text of the whole thesis, papers included.




The *"world cloud"* shows the main topics afforded and testify the high level of cross branch approach needed for realization of such devices.

An extensive (15 pages) and clear introduction chapter explains to expert or common readers the principles of biosensor science introducing all the needed elements for understanding the

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following experimental parts of the thesis. Very useful for readability the one page list of abbreviations used in the thesis and in the papers.

Objectives are declared clearly and schematically without sacrificing detailed description of milestone of the work.

In my opinion the section "Materials and methods" is well described with all the needed details for easy reproduction of all the experiments and results. This section shows a very high level of cross disciplinarity between biology, chemistry, physic and the use of computer aided simulations or technological processes (microelectronics, nanotechnology)

Results and discussion are very interesting. Noteworthy are the results about the thickness of the PSII layer in the herbicide biosensor, the use of the sol-gel as an alternative to cross linking of the photosystem II withBSA- glutharaldehyde. These results were optimized for herbicides monitoring. Labeling by use of silver nanoparticles encapsulated in dendrimer structures is an original approach. Interesting results were obtained in design of microfluidics and in the use of magnetic beads for immobilization in microfluidic devices, too.

Conclusions perfectly fit the objectives declared previously.

Bibliographic references are complete and updated in all the chapters of the thesis.

The attached papers are confident with the described results and the role of Mr.Marcel Stofik in the work done.

In conclusion I read the thesis with big pleasure several times because it is wrritten very well and clear, because the objectives are relevant and ambitious and because the results fitted the goals promised previously.

The opponent: Roberto Pilloton

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Rome 02/09/2012

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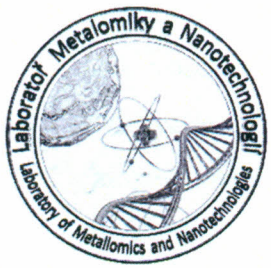


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**Dissertation Assessment on Thesis of Mgr. Marcel Štofik**

Dissertation Thesis of Mgr. Marcel Štofik, which experimental part has been performed on Institute of Microbiology of the Academy of Sciences in Třeboň and Faculty of Science of University of South Bohemia in České Budějovice, entitled "Biosensors for Environmental Monitoring and Biomedical Applications" attempts to introduce and briefly summarize the basics on sensors and biosensors, whereas the results are aimed at suggestion and preparation of biosensors based on advanced materials for environmental and biomedical purposes.

Dissertation Thesis is wide-ranging (186 pages) with valuable and up-to date reference list. It should be stated without any doubts that the thesis is well written without fatal errors or gaps. Introduction as theoretical part of the thesis is 16 pages long with short overview on sensors and biosensors with respect to their utilization for environmental and biomedical purposes. The introduction is short but concise and fulfils its role of reader introducing to the topic of interest. The results are divided into two parts. The first is devoted to electrochemical biosensing of herbicides. The second part focuses on synthesis of metal labels and their using for electrochemical biosensing. Moreover, microfluidic chip as well as magnetic particles based separation is successfully tested.

Considering the fact that this is interesting topic with great development in this field, postgraduate handled the topic very well and have published two papers in highly impacted journals under perfect supervision. In addition, three papers have been prepared and one Utility model successfully utilized.

With respect to the fact that results have been internationally reviewed, there are no doubts about high quality of the presented results. Nevertheless, I have the following questions

and comments for discussion:

- Have you ever listened about Palecek's Adsorptive Transfer Striping Technique as a modification to basic stripping approaches? If so, please, can you consider some of the main advantages of this method for your immune-based biosensing?
- Do you think that there could be suggested well-portable device for detection of not only herbicides bot also for products of their chemical interactions with biota?
- Is it possible to encapsulate other metal nanoparticles into PAMAM-OH? If so, would it be possible to use the same protocol or with some changes? Moreover, why did you choose silver nanoparticles?
- There are few papers under preparation. To which journals do you plant to submit them?
- What are future perspectives of your work? Are you interested in measuring of some real sample from patients considering your biosensing device, which is opened for various purposes?

Above mentioned questions and suggestions should be considered as a base for discussion, because the thesis is of high quality. Based on the quality of the thesis, I would like to recommend Dissertation Thesis of Mgr. Marcel Štofik to be defended with evaluation "excellent".

Brno, 31st August, 2012

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